APPENDIX B

CLAIMS PENDING IN USSN 09/101,283 WITH ENTRY OF THIS AMENDMENT

1. A protein released by a mammalian fetal trophoblast cell or a chorionic villus wherein the level of release is substantially changed when the cell or villus is grown under hypoxic conditions characterized by a partial pressure of oxygen (pO_2) of 14 mm of mercury (mm Hg), wherein said protein is selected from the group of proteins consisting of:

(a) Protein A having a molecular weight of about 21 kDa and a pI of 6.0 wherein the release of said protein, under hypoxic conditions is increased;

(b) Protein B having a molecular weight of about 22 kDa and a pI of 7.0 wherein the release of said protein, under hypoxic conditions is increased;

(c) Protein C having a molecular weight of about 23 kDa and a pI of 7.5 wherein the release of said protein, under hypoxic conditions, is increased;

(d) Protein D having a molecular weight of about 55 kDa and a pI of 8.5 wherein the release of said protein, under hypoxic conditions, is increased;

(e) Protein E having a molecular weight of about 62 kDa and a pI of 5.5 wherein the release of said protein, under hypoxic conditions, is increased;

(f) Protein F having a molecular weight of about 40 kDa and a pI of 4.5 wherein the release of said protein, under hypoxic conditions, is decreased;

(g) Protein G having a molecular weight of about 67 kDa and a pI of 6.5 wherein the release of said protein, under hypoxic conditions, is decreased;

(h) Protein H having a molecular weight of about 75 kDa and a pI of 9.0 wherein the release of said protein, under hypoxic conditions, is decreased;

(i) A protein of spot number 2 comprising an amino acid sequence selected from the group consisting of sequence 1, and sequence 2 as shown in Table 2;

(j) A protein of spot number 3 comprising an amino acid sequence selected from the group consisting of sequence 3, sequence 4, sequence 5, and sequence 6 as shown in Table 2;

(k) A protein of spot number 5 comprising amino acid sequence number 7 as shown in Table 2;

(l) A protein of spot number 7 comprising amino acid sequence number 8 as shown in Table 2;

(m) A protein of spot number 10 comprising an amino acid sequence selected from the group consisting of sequence sequence 12, and sequence 13 as shown in Table 2;

(n) A protein of spot number 11 comprising an amino acid sequence selected from the group consisting of sequence 14, sequence 15, sequence 16, sequence 17, and sequence 18 as shown in Table 2; and

(o) A protein of spot number 20 comprising an amino acid sequence selected from the group consisting of sequence 21, and sequence 22 as shown in Table 2; and

(p) A human apolipoprotein A-1.

2. A protein of claim 1, wherein the protein is selected from the group consisting of:

(a) Protein A having a molecular weight of about 21 kDa and a pI of 6.0 wherein the release of said protein, under hypoxic conditions, is increased;

(b) Protein B having a molecular weight of about 22 kDa and a pI of 7.0 wherein the release of said protein, under hypoxic conditions, is increased;

(c) Protein C having a molecular weight of about 23 kDa and a pI of 7.5 wherein the release of said protein, under hypoxic conditions, is increased;

(d) Protein D having a molecular weight of about 55 kDa and a pI of 8.5 wherein the release of said protein, under hypoxic conditions, is increased; and,

(e) Protein E having a molecular weight of about 62 kDa and a pI of 5.5 wherein the release of said protein, under hypoxic conditions, is increased.

3. Withdrawn from consideration.

4. Withdrawn from consideration.

5. A method of culturing human fetal trophoblast cells or chorionic villi under hypoxic conditions, said method comprising culturing the trophoblast cells or chorionic villi under an atmosphere comprising less than about 20% oxygen.

6. A method of claim 5, wherein the method further comprises measuring the release of a protein selected from the group consisting of:

(a) Protein A having a molecular weight of about 21 kDa and a pI of 6.0 wherein the release of said protein, under hypoxic conditions, is increased;

(b) Protein B having a molecular weight of about 22 kDa and a pI of 7.0 wherein the release of said protein, under hypoxic conditions, is increased;

(c) Protein C having a molecular weight of about 23 kDa and a pI of 7.5 wherein the release of said protein, under hypoxic conditions, is increased;

(d) Protein D having a molecular weight of about 55 kDa and a pI of 8.5 wherein the release of said protein, under hypoxic conditions, is increased;

(e) Protein E having a molecular weight of about 62 kDa and a pI of 5.5 wherein the release of said protein, under hypoxic conditions, is increased;

(f) Protein F having a molecular weight of about 40 kDa and a pI of 4.5 wherein the release said protein, under hypoxic conditions, is decreased;

(g) Protein G having a molecular weight of about 67 kDa and a pI of 6.5 wherein the release of said protein, under hypoxic conditions, is decreased; and

(h) Protein H having a molecular weight of about 75 kDa and a pI of 9.0 wherein the release of said protein, under hypoxic conditions, is decreased;

(i) A protein of spot number 2 comprising an amino acid sequence selected from the group consisting of sequence 1, and sequence 2 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is decreased;

(j) A protein of spot number 3 comprising an amino acid sequences selected from the group consisting of sequence 3, sequence 4, sequence 5, and sequence 6 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is decreased;

(k) A protein of spot number 5 comprising amino acid sequence number 7 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is increased;

(l) A protein of spot number 7 comprising amino acid sequence number 8 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is increased;

(m) A protein of spot number 10 comprising an amino acid sequence selected from the group consisting of sequence 12, and sequence 13 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is increased;

(n) A protein of spot number 11 comprising an amino acid sequence selected from the group consisting of sequence 14, sequence 15, sequence 16, sequence 17, and sequence 18 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is decreased; and

(o) A protein of spot number 20 comprising an amino acid sequence selected from the group consisting of sequence 21, and sequence 22 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is increased; and

(p) A human apolipoprotein A-1 wherein the release of said protein, under hypoxic conditions, is increased

where alteration in release of the proteins as described above indicates that said cell is characteristic of a trophoblast in an abnormal placental interface.

7. A method of detecting hypoxic cytotrophoblast cells or hypoxic chorionic villi, said method comprising measuring the release of a protein selected from the group consisting of:

(a) Protein A having a molecular weight of about 21 kDa and a pI of 6.0 wherein the release of said protein, under hypoxic conditions, is increased;

(b) Protein B having a molecular weight of about 22 kDa and a pI of 7.0 wherein the release of said protein, under hypoxic conditions, is increased;

(c) Protein C having a molecular weight of about 23 kDa and a pI of 7.5 wherein the release of said protein, under hypoxic conditions, is increased;

(d) Protein D having a molecular weight of about 55 kDa and a pI of 8.5 wherein the release of said protein, under hypoxic conditions, is increased;

(e) Protein E having a molecular weight of about 62 kDa and a pI of 5.5 wherein the release of said protein, under hypoxic conditions, is increased;

(f) Protein F having a molecular weight of about 40 kDa and a pI of 4.5 wherein the release of said protein, under hypoxic conditions, is decreased;

(g) Protein G having a molecular weight of about 67 kDa and a pI of 6.5 wherein the release of said protein, under hypoxic conditions, is decreased; and

(h) Protein H having a molecular weight of about 75 kDa and a pI of 9.0 wherein the release of said protein, under hypoxic conditions, is decreased;

(i) A protein of spot number 2 comprising an amino acid sequence selected from the group consisting of sequence 1, and sequence 2 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is decreased;

(j) A protein of spot number 3 comprising an amino acid sequences selected from the group consisting of sequence 3, sequence 4, sequence 5, and sequence 6 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is decreased;

(k) A protein of spot number 5 comprising amino acid sequence number 7 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is increased;

(l) A protein of spot number 7 comprising amino acid sequence number 8 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is increased;

(m) A protein of spot number 10 comprising an amino acid sequence selected from the group consisting of sequence 12, and sequence 13 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is increased;

(n) A protein of spot number 11 comprising an amino acid sequence selected from the group consisting of sequence 14, sequence 15, sequence 16, sequence 17, and sequence 18 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is decreased; and

(o) A protein of spot number 20 comprising an amino acid sequence selected from the group consisting of sequence 21, and sequence 22 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is increased; and

(p) A human apolipoprotein A-1 wherein the release of said protein, under hypoxic conditions, is increased;

wherein the release of the protein is increased or decreased relative to identical cells grown under identical culture conditions but under normal oxygen conditions.

8. A method of claim 6, wherein the measurement is by direct determination of the protein.

9. A method of claim 6, wherein the determination comprises the step of binding an antibody to the protein and determining the quantity of bound antibody present in a sample relative to the quantity of antibody bound to protein obtained from normoxic trophoblasts or normoxic chorionic villi.

10. A method of claim 6, wherein the determination comprises detecting mRNA encoding any of the proteins and determining if the level of mRNA has changed relative to similarly treated normoxic cells.

11. A method for detecting an abnormal placental function said method comprising :[by]

analyzing a biological sample from a pregnant mammal for abnormal release of a protein, wherein said abnormal release is selected from the group consisting of:

(a) Protein A having a molecular weight of about 21 kDa and a pI of 6.0 wherein the release of said protein, under hypoxic conditions, is increased;

(b) Protein B having a molecular weight of about 22 kDa and a pI of 7.0 wherein the release of said protein, under hypoxic conditions, is increased;

(c) Protein C having a molecular weight of about 23 kDa and a pI of 7.5 wherein the release of said protein, under hypoxic conditions, is increased;

(d) Protein D having a molecular weight of about 55 kDa and a pI of 8.5 wherein the release of said protein, under hypoxic conditions, is increased;

(e) Protein E having a molecular weight of about 62 kDa and a pI of 5.5 wherein the release of said protein, under hypoxic conditions, is increased;

(f) Protein F having a molecular weight of about 40 kDa and a pI of 4.5 wherein the release of said protein, under hypoxic conditions, is decreased;

(g) Protein G having a molecular weight of about 67 kDa and a pI of 6.5 wherein the release of said protein, under hypoxic conditions, is decreased; and,

(h) Protein H having a molecular weight of about 75 kDa and a pI of 9.0 wherein the release of said protein, under hypoxic conditions, is decreased;

(i) A protein of spot number 2 comprising an amino acid sequence selected from the group consisting of sequence 1, and sequence 2 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is decreased;

(j) A protein of spot number 3 comprising an amino acid sequences selected from the group consisting of sequence 3, sequence 4, sequence 5, and sequence 6 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is decreased;

(k) A protein of spot number 5 comprising amino acid sequence number 7 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is increased;

(l) A protein of spot number 7 comprising amino acid sequence number 8 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is increased;

(m) A protein of spot number 10 comprising an amino acid sequence selected from the group consisting of sequence 12, and sequence 13 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is increased;

(n) A protein of spot number 11 comprising an amino acid sequence selected from the group consisting of sequence 14, sequence 15, sequence 16, sequence 17, and sequence 18 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is decreased; and

(o) A protein of spot number 20 comprising an amino acid sequence selected from the group consisting of sequence 21, and sequence 22 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is increased; and

(p) A human apolipoprotein A-1 wherein the release of said protein, under hypoxic conditions, is increased.

12. A method of claim 10, wherein said abnormal placental function is a symptom of a disease of pregnancy selected from the group consisting of threatened abortion, intrauterine growth retardation, gestational trophoblast diseases including molar pregnancy, choriocarcinoma, placental site tumors, ectopic pregnancy, proteinuria, pregnancy induced hypertension and preeclampsia.

13. A method of claim 11, wherein said disease of pregnancy is preeclampsia.

14. A method of screening for agents that mitigate the effects of an abnormal maternal-placental interface, said method comprising:

(i) culturing cytotrophoblasts under hypoxic conditions in the presence of said agent; and

(ii) assaying for changes in the phenotype of said hypoxic trophoblasts relative to hypoxic trophoblasts cultures without the presence of said agent.

15. The method of claim 13, wherein said assaying comprises measuring the invasiveness of said trophoblasts.

16. The method of claim 13, wherein said assaying comprises measuring the changes in the levels of release of proteins expressed by said trophoblasts.

17. The method of modeling, in vitro, an abnormal maternal-placental interface, said method comprising culturing trophoblast cells or chorionic villi in a hypoxic environment.

18. The method of claim 16, wherein said hypoxic environment comprises an atmosphere comprising less than about 20% oxygen.

19. A method for identifying proteins that are indicative of metastasis said method comprising:

(i) raising cytotrophoblasts under hypoxic conditions; and

(ii) detecting proteins that demonstrate an altered release level as a result of said hypoxic conditions; and,

(iii) determining if said proteins are present in metastatic cells.

20. A method of claim 18, wherein the determining is done by immunoassay using antibodies specific for at least one of the proteins of step ii.

21. A method for identifying proteins that are indicative of an abnormal maternal placental interface said method comprising:

(i) culturing cytotrophoblasts under hypoxic conditions; and

(ii) detecting proteins that demonstrate an altered release level as a result of the hypoxic conditions.

22. Withdrawn from consideration.